Nitrogen rhizodeposition by wheat under different tillage systems in a rainfed Vertisol

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Abstract

Roots and the nutrients that they deposit into the soil are a natural source of N for a current crop, or a subsequent crop in the case of rotations. Despite the importance of this process, there are few field studies of rhizodeposition due to methodological difficulties. A two-year field study was conducted on a typical Mediterranean rainfed Vertisol to determine the effects of the tillage system on N rhizodeposition in wheat (*Triticum aestivum* L.). The tillage treatments were no-tillage (NT) and conventional tillage (CT). Wheat plants were labelled *in situ* with ^{15}N using a leaf feeding method. The total amount of N that was deposited by the roots in the full soil profile (0-75 cm) over two years was an average of 93 kg ha^{-1}. The N derived from rhizodeposition (NdfR) represented 40% of the total N content and 82% of belowground N for the wheat plant. The NdfR was higher under NT only in the first layer of the soil (0-15 cm). Fifty percent of the NdfR in the soil profile was observed within the first 15 cm, increasing to 74% within the first 30 cm. The amount of NdfR can be important for understanding farming systems and improving their management, especially the application of fertilizers.

Keywords no tillage; conventional tillage; ^{15}N labelling; leaf feeding; N recovery; N enrichment

1. Introduction

The fertility of soils in Mediterranean rainfed agrosystems is generally not optimum for the maximum yield of a crop. Therefore, all of the sources of nutrients to the plant are important. The rhizodeposition of plants provides a natural source of nitrogen, although there is some disagreement regarding its exact definition. Some authors, such as Kuzyakov and Schneckenberger (2004) and Wichern et al. (2008), believe that fine roots, root fragments and decaying roots should be included in the term. Others, such as Jensen (1996) and Frank and Groffman (2009), only consider the exudate, secretions, lysates, sloughed cells and mucilage from the roots as rhizodeposits. Rasmussen (2011) indicated that an operational definition of rhizodeposition should include N from the turnover of root hairs, fine roots and minor root fragments but would exclude N from the turnover of decaying roots and larger parts of the root system because it is implied that these plant tissues are no longer living.

Janzen (1990) reported that the N that was deposited in the rhizosphere appeared to be much more labile than indigenous and fertiliser-derived organic N. This effect, coupled with the relatively large amounts of N root deposits in the soil, suggests that rhizodeposition may contribute significantly to the N fertility of soils (Janzen, 1990). Furthermore, the amount of N-containing substance released from the roots can affect the microbial activity in the rhizosphere and the acquisition of other nutrients by plants and/or microorganisms (Palta et al., 1991).

Quantitatively estimating rhizodeposition is crucial for understanding soil N turnover processes (Mayer et al., 2003) and the availability of N for subsequent crops (Mayer et al., 2003, 2004). There are few quantitative studies of N rhizodeposition under field conditions due to the complexity inherent in such studies. Although there are various methods for *in situ* studies, they are subject to problems that demonstrate the difficulty of calculating rhizodeposition. Currently, the techniques used to estimate the amount of N that is derived from rhizodeposition (NdfR) are based on labelling aboveground plant parts with the stable N isotope ^{15}N.

Rhizodeposition is influenced by abiotic and biotic factors (Jones et al., 2004). The tillage system can modify some of these factors, such as bulk density, soil strength, water retention capacity and the degradation of macropore space and pedality. López-Bellido et al. (2011) studied the influence of tillage systems on the amount of N that was deposited by the roots of legumes under field conditions.

The NdfR values that have been obtained from wheat in controlled experiments vary widely (Janzen, 1990; Janzen and Bruinsma, 1993; Merbach et al., 2000; Khan et al., 2002) due to the difference in experimental conditions, methods and environmental factors (Wichern et al., 2008). According to Janzen (1990), more than one-third of the N that is assimilated by a wheat crop may reside below ground at the time of harvest because the N deposition by wheat is closely related to the plant's total N content (Janzen and Bruinsma, 1993).

The lack of uniformity in the ^{15}N distribution within a plant when using a single pulse has been widely discussed (Götz and Herzog, 2000). The accumulation of N in various parts of the plant depends on the type and number of sinks that are present during labelling. However, the ^{15}N enrichment of the roots (assuming homogeneity throughout the root system) would be

sufficient to estimate the NdfR using a single pulse (Rochester et al., 1998). According to Wichern et al. (2008) and Fustec et al. (2010), continuous labelling enables a more homogeneous and uniform label, resulting in a representative estimate of the quantity of root-derived compounds (Jensen, 1996). However, a complete, continuous labelling of plants is difficult to achieve, especially under field conditions.

The objective of this study was to determine the effects of the tillage system on N rhizodeposition and N derived from wheat roots when grown in a Vertisol soil under Mediterranean, rainfed conditions using an *in situ* ^{15}N labelling technique.

2. Materials and methods

2.1.Site and experimental design

The field experiment was conducted in Córdoba, southern Spain (37º46'N, 4º31'W, 280 m a.s.l.), on a Vertisol (Typic Haploxererts) typical of the Mediterranean region where rainfed cropping is the standard practice. The study took place over a 2 year period (2007–2008 and 2008–2009) within the framework of a long-term experiment, named "Malagón", which began in 1986. The properties of the Vertisol that was used in the field experiments were documented by López-Bellido et al. (1997). The main plots were the tillage systems: no tillage (NT) and conventional tillage (CT). The experimental design was a randomised complete block with three replications. The wheat was grown in a 2 year rotation with sunflower (*Helianthus annuus* L.). The rotation was duplicated in a reverse crop sequence to obtain data for both crops on a yearly basis. Only the wheat plots were fertilised with 100 kg N ha^{-1} and 65 kg P ha^{-1}.

2.2.Weather conditions

The rainfall distribution differed between the two years. The precipitation was 465 mm in 2007-2008 and 520 mm in 2008-2009. In 2008-2009, the distribution of rainfall during the growing season was more homogeneous than in the previous year (Fig. 1). The mean temperature was similar in both years, while the maximum temperature for the months of September through February in 2007-2008 was higher than that in 2008-2009 (Fig. 1).

2.3.Crop management

The no-tillage plots were sown with a no-till seed drill. Weeds were controlled with glyphosate [[N-(phosphonomethyl) glycine] + MCPA [(4-chloro-2-methylphenoxy) acetic acid]] at a rate of 0.5 + 0.5 l a.i. ha^{-1} prior to planting. The conventional tillage treatment included mouldboard ploughing, disk harrowing and/or vibrating tine cultivation to prepare a proper seedbed. Wheat (*Triticum aestivum* L. cv. Gazul) was planted in 18 cm rows in early December at a seeding rate of 150 kg ha^{-1}. At harvest, a 0.5 m^2 area at the centre of each wheat plot was sampled. From this sample, the aboveground biomass was measured by drying plants at 80 ºC to a constant weight. Each year, the wheat grains were harvested in June using a Nurserymaster Elite Plot Combine (30 m^2 per plot).

2.4.Plant labelling and sampling

PVC columns that were 20 cm in diameter (314 cm^2) were inserted into the soil to a depth of 30 cm in a uniform area of the crop. The plants within these frames were thinned to an optimal plant density of 7-8 plants per column (equivalent to 230 plants m^{-2}). To determine the N

rhizodeposition, all of the plants in each column were labelled in situ with a ^{15}N urea solution. At the early stem elongation stage, the cut tip of a fully expanded leaf from each plant was immersed in 1.5 ml of 0.5% ^{15}N-enriched urea solution (98% atom. ^{15}N) and placed in a plastic vial that was attached to the stem. Each vial was covered with a rubber stopper to prevent evaporative losses or the addition of water during rainfall. The leaves were placed in the solution for 4 days, and, subsequently, the remaining ^{15}N urea in each vial was recovered and measured.

The plants inside the columns were harvested at physiological maturity, and the shoots and roots were separated. Fallen leaves were collected regularly from a cloth that was laid over the soil surface and were then added to the harvested material. All of the material was dried at 70 ºC for 48 h and then weighed and ground. After harvest, three cylindrical soil cores were sampled from the columns at depths of 0–15, 15 –30, 30–45, 45–60 and 60–75 cm using a 5 cm diameter bi-partite root auger (Eijkelkamp plant root auger, Amsterdam, the Netherlands). Soon after sampling, the soils were frozen at −30 °C to avoid root decomposition. All of the visible roots from each soil core were subsequently removed by hand from the soil, and the soil was sieved. Roots and root fragments were manually collected during the sieving process and washed with distilled water. The soil that adhered to the fresh roots was separated from the roots and mixed with the bulk soil. All of the recovered roots were subsequently dried at 70 ºC to a constant weight. Subsamples of the recoverable roots were muffled at 550 ºC for 3 h to correct their N analyses for soil contamination.

In this study, N rhizodeposition is defined as the release of all types of N compounds that are lost from living plant roots after the removal of recoverable roots. Aboveground plant samples, root material and soil were finely pulverised and analysed for their ^{15}N isotopic abundances

using a Carlo Erba 1108 Elemental Analyzer that was coupled to a VG Isochrom isotope ratio mass spectrometer in continuous flow. The total N concentration was analysed using the Dumas combustion method (EA 3000 Eurovector SpA, Milan, Italy).

2.5.Calculations

The ^{15}N recovery (N_R) by area and percentage was calculated following Hauck and Bremner (1976):

$$N_R = N_t \times \frac{c-b}{a-b} \quad \text{and} \quad \%N_R = \frac{N_R}{f} \times 100$$

where, Nt is the total plant N at maturity, in kg ha^{-1}; a is the atom% ^{15}N in the fertiliser; b is the atom% ^{15}N in the unfertilised plant; c is the atom% ^{15}N in the fertilised plant; and f is the fertiliser application rate, in kg N ha^{-1}, that was applied to the crop.

The percentage of the total plant N that was derived from ^{15}N fertiliser (N_F) was calculated:

$$N_F = \frac{N_R}{N_t} \times 100$$

The total ^{15}N recovery in the soil plant system was calculated as the sum of the ^{15}N content in the stems and leaves (including any leaves that were collected from the ground), grains, macro roots and rhizodeposits. To perform these calculations, the following assumptions were made: (i) all excess ^{15}N that was detected in the soil originated from the ^{15}N-enriched belowground biomass of wheat and (ii) the recovered roots that were isolated from each soil layer were representative of the N concentration and ^{15}N enrichment of all of the root tissues in that layer. Assuming that the N that was deposited had the same ^{15}N enrichment as the sampled macro root, the rhizodeposition relative to the total N in the bulk and rhizosphere soils was calculated using the following equation (Janzen and Bruinsma, 1989):

$$\%NdfR = \left[\text{atom \% } ^{15}\text{N excess (soil)} / \text{atom \% } ^{15}\text{N excess (roots)}\right] \times 100$$

where the excess atom% ^{15}N (^{15}N enrichment) in the roots was calculated as the atom% ^{15}N in the recovered roots minus the atom% ^{15}N in atmospheric N_2. The ^{15}N enrichment of the soil was calculated as the atom% ^{15}N from the soil inside the steel frames minus the average natural ^{15}N enrichment of the soil N from the harvest of the wheat plots that were not ^{15}N enriched (0.3691 atom% ^{15}N) (Schmidtke, 2005).

The total amount of rhizodeposited N (kg N ha^{-1}) was calculated:

$$\text{NdfR} = N_S \times \%NdfR / 100$$

where Ns is the total amount of N in the soil or soil layer, expressed in kg N ha^{-1}.

The average values for N content that were used in the calculations were 0.08, 0.079, 0.072 and 0.061% for conventional tillage and 0.089, 0.078, 0.071 and 0.07% for no-tillage in the 0-15, 15-30, 30-60 and 60-75 cm profiles, respectively.

2.6.Statistical analyses

The year was considered as a random variable in this study due to the unpredictable weather conditions in the Mediterranean region (Gómez and Gómez, 1984). Nitrogen rhizodeposition (percentage and kg ha^{-1}) and the rest of the parameters were used in an analysis of variance (ANOVA) with a randomised complete block design that combined the years and soil depths

using the error term described by McIntosh (1983). When the treatment methods had significant effects, they were compared using Fisher's protected least significant difference (LSD) test at $P \leq 0.05$. The analyses of variance were performed using Statistix 8.1 (Analytical Software 2007) to determine the treatment effects.

3. Results

3.1.Biomass production and N uptake

The production of both aboveground biomass (grain and straw) and roots was higher in 2008-2009, with values approximately double those of 2007-2008 (Table 1). The N uptake in the aboveground biomass, rhizodeposits and roots was also higher in 2008-2009 compared to the previous year (Table 1). The majority of the aboveground assimilated N was recovered in grains (Table 1 and 2). The N content of the roots accounted for only 17% of the belowground N content and 8% of the total N content of the plant.

The tillage system influenced the wheat biomass and the amount of N in the grain and straw (Table 1). The biomass of grain and straw was higher in the NT plots, as was the amount of N in the grain. However, the amount of N in the straw was higher in the CT plots (Table 1).

3.2.Rhizodeposition of N in the soil

The quantity of the NdfR in the soil profile (0-75 cm) was significantly higher in 2008-2009 than in 2007-2008 (Table 1). The NdfR was not influenced by the tillage system (Table 1). The percentage of NdfR was 41% of the total N uptake for 2007-2008 and 38% for 2008-2009

(Table 1). Wheat rhizodeposition contributed 82% of the belowground N (Table 2). The NdfR:straw biomass was 0.016, and the NdfR:root biomass was 0.06.

Differences between tillage systems were only observed in the first 15 cm of soil in 2007-2008 (Fig. 2), when the NT plots had a higher NdfR (30 kg ha^{-1}) than the CT plots (53 kg ha^{-1}). The first 15 cm of soil depth contained more NdfR than the other depths for both years and tillage systems. In 2007-2008, there were no significant differences below 15 cm for the NT plots or below 30 cm for the CT plots. However, in 2008-2009, the NdfR gradually decreased down to 45 cm in the CT plots and down to 60 cm in the NT plots. The 15 cm of topsoil contained fifty percent of the total NdfR in the whole soil profile (0-75 cm). This percentage increased to 74% in the first 30 cm of soil (Fig. 2).

3.3. ^{15}N recovery and distribution

The ^{15}N recovery by wheat was not influenced by either the year or the tillage system, with mean values of 48% in grain, 13% in straw, 2.8% in roots and 15.5% in the rhizodeposits; thus, approximately one quarter of the total ^{15}N recovery was detected belowground (Table 2). The wheat crop recovered 79.3% of the total ^{15}N that was applied (Table 2).

The ^{15}N enrichment was not influenced by the tillage system (Table 2). Wheat had the highest ^{15}N enrichment in grains (1.17 atom% ^{15}N excess), followed by straw (1.06 atom% ^{15}N excess), roots (0.35 atom% ^{15}N excess) and soil (0.006 atom% ^{15}N excess) (Table 2). Forty-three percent of the ^{15}N enrichment in the roots occurred within the first 15 cm of soil, increasing to 67% within the first 30 cm (Fig. 3).

The total N was distributed between the grain and rhizodeposits, while the ^{15}N was distributed predominantly in the grain (Table 3).

4. Discussion

The amount of belowground N accounted for 50% of the plants' total N throughout the experiment. Rroço and Mengel (2000) and Khan et al. (2002) reported that the proportion of net assimilated N that is transferred belowground varies between 16% and 60% in cereals, with an average of 36%. Janzen (1990) specifically reported that more than one third of the N that is assimilated by wheat crops may reside belowground at the time of harvest, with a median of 36%. Janzen and Bruinsma (1989) reported that belowground N ranged between 44% - 53% of the total N that was assimilated by wheat plants.

Similar to the results obtained by Wichern et al. (2007) in oats, more than a third of the total N in the wheat plant was observed in the grain in our experiment. Our results for the amount of N in the roots differ from those of other studies that have been conducted under laboratory conditions. However, Wichern et al. (2007), who conducted experiments with soil columns under field conditions, reported a percentage of N uptake in oat roots in relation to total N content that was similar to ours (9%).

The above- and belowground biomass production was higher in 2008-2009, when there was more and better distributed rainfall during the growing season. Consequently, the amount of N in different parts of the plant was also higher in 2008-2009. That amount of nitrogen that was derived from rhizodeposition (NdfR) was also higher this year because there was a greater biomass of wheat roots. According to de Graaf et al. (2007), the amount of rhizodeposition is

proportional to root production. For the same reason, there was no tillage system effect on either the root biomass or NdfR.

The NdfR for the net assimilated N in wheat varies widely, between 4.3% and 56%, according to Merbach et al. (2000) and Khan et al. (2002). These variations are due to the use of different methods for labelling plants with ^{15}N and varying experimental conditions, as noted by Wichern et al. (2007). The NdfR values in our study are within this range, although most studies report values between 5% and 20% (Janzen and Bruinsma, 1989, 1993; Janzen, 1990; Merbach and Schulze, 1998). These studies were conducted in pots under controlled or laboratory conditions, where it is easier to separate and recover the majority of root fragments. In contrast, under field conditions, it is difficult to recover all of the root fragments, resulting in an overestimation of NdfR. We must add that because of the specificity of the pot experiments, which have a relatively small soil volume per plant, the root density is greater. Therefore, the root:shoot ratio in field conditions will shift in favour of the roots compared to pot experiments, leading to greater rhizodeposition under field conditions, as noted by Mayer et al. (2003).

In a study by López-Bellido et al. (2011) under the same Mediterranean, rainfed conditions in a Vertisol soil, the values of N assimilation and NdfR in kg ha^{-1} for faba bean and chickpea crops were very similar to those of our study with wheat. However, the NdfR:shoot biomass and NdfR:root biomass in both legumes were five times higher than those obtained in this experiment with wheat; the same results were found for the amount of N. Therefore, wheat has less NdfR per unit of root and shoot biomass than legumes; while wheat provided 60 g of NdfR per kg of root, legumes provided just 330 g (López-Bellido et al., 2011). Consequently, the amount of N deposited per plant in the soil is higher for legumes than for wheat due to a higher total N content. The similarity of NdfR in kg ha^{-1} between the two crops can be attributed to

the higher root density of wheat plants compared with pulses. Wichern et al. (2008) also reported NdfR values per plant that were much higher for faba beans and chickpeas than wheat.

There were greater differences in NdfR in 2008-2009 at varying soil depths. In this year, rainfall was higher and more evenly distributed. According to Muñoz-Romero et al. (2010ab), this rainfall pattern could cause the root system to develop greater depth, while in a year with less precipitation that is less evenly distributed (2007-2008), roots tend to grow closer to the surface. The first 30 cm of soil contained 75% of the NdfR, a finding consistent with that reported by Wichern et al. (2007). In 2007-2008, the top 15 cm of the soil had more NdfR in the CT plots than in the NT plots because the CT plots had a higher root biomass, as reported by Muñoz-Romero et al. (2010a).

Our ^{15}N recovery values are higher than those obtained by Wichern et al. (2007) for oats and similar to those obtained by Mayer et al. (2003) for legumes. According to Wichern et al. (2007), the loss of ^{15}N is partly a consequence of the present experimental conditions in the field, which may have caused losses due to unrecoverable shed leaves and experimental errors in the mass balance between the ^{15}N that was recovered in the various plant parts and the amounts that were applied. Approximately 60% of the total N recovery of the plant was located in the grain, which is consistent with the results of Wichern et al. (2007), who obtained a similar result in an oat crop at maturity. The ratio of recovered ^{15}N to N in the different plant fractions (Table 3) was between 0.3 and 1.7. The ratio was below 1.0 in the belowground biomass and above 1.0 in the aboveground biomass, according to Wichern et al. (2007), indicating preferential enrichment of the aboveground biomass.

The value of crop enrichment was within the range obtained by Wichern et al. (2007) for oats. In addition, the fractions of grain and shoots were preferentially enriched, while enrichment in the roots was lower for different shoot labelling methods (López-Bellido et al., 2011; Wichern et al., 2007; Yasmin et al., 2006; Jensen, 1996; Janzen and Bruinsma, 1989). In addition, Palta et al. (1991) reported that root enrichment was lower than shoot enrichment in wheat using the same leaf feeding method that was used in the present experiment.

5. Conclusions

Under field conditions in a rainfed, Mediterranean cropping system, 40% of the total N content of the plant and 82% of the belowground N was derived from rhizodeposition. Wheat contributes a large amount of N derived from rhizodeposition to the soil due to its dense and well-developed root system. The average NdfR over the two years was 93 kg ha^{-1}; in years with more and better distributed rainfall, NdfR was greater. This contribution was higher in the first 30 cm of the soil due to the higher root biomass in this layer. The no-tillage plots had higher NdfR than the conventional tillage plots only within the first 15 cm of the soil in 2007-2008.

Previously, the majority of studies of NdfR for wheat have been performed under controlled conditions. Additional field studies are needed to determine the actual contribution of NdfR for cereal crops. An understanding of the amount of NdfR that is available and its distribution within the soil profile under field conditions is of great importance in determining the N fertilizer needs of subsequent crops.

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Table 1. Above- and below-ground biomass (0-75 cm) and N uptake as affected by year and tillage system (NT, no tillage; CT, conventional tillage) in wheat.

	Biomass (kg ha^{-1})				N uptake (kg ha^{-1})				
	Grain	Straw	Roots	Total	Grain	Straw	Roots	NdfR	Total
Year									
2008	2666b[†]	4034b	1092b	7792b	70b	22b	15b	75b	182b
2009	5282a	8160a	2163a	15605a	125a	35a	25a	111a	296a
Tillage									
NT	4217a	6380a	1722a	12319a	101a	27b	21a	101a	250a
CT	3730b	5815b	1533a	11078b	93b	30a	20a	85a	228b

[†]Within treatment means followed by the same letter are not significantly different at P<0.05 according to LSD.

Table 2. Enrichment of ^{15}N and ^{15}N recovery in plant parts of wheat.

	^{15}N excess (atom.%)				Recovery of ^{15}N label (%)				
	Grain	Straw	Roots	Soil	Grain	Straw	Roots	NdfR	Total
Year									
2008	1.1a[*]	1.3a	0.4a	0.007a	40.5a	14.8a	1.9a	12.7a	69.9a
2009	1.2a	0.8b	0.3a	0.005a	56.4a	10.7a	3.7a	18.1a	88.9a
Tillage									
NT	1.1a	1.1a	0.4a	0.007a	48.8a	13.3a	3.0a	17.5a	82.6a
CT	1.3a	1.0a	0.3a	0.006a	48.0a	12.1a	2.6a	13.3a	76.0a

[*] Within columns and treatments (year and tillage) means followed by the same letter are not significantly different at P<0.05 according to LSD
NdfR: nitrogen derived from rhizodeposition.

Table 3. Distribution of the ^{15}N label, and distribution of total N content in wheat.

	Distribution of recovered ^{15}N (%)					Distribution of total N (%)				
	Grain	Straw	Roots	NdfR	Total	Grain	Straw	Roots	NdfR	Total
Year										
2008	57.9	21.2	2.7	18.2	100	38.5	12.1	8.2	41.2	100
2009	63.4	12.0	4.2	20.4	100	42.2	11.7	8.5	37.5	100
Tillage										
NT	59.1	16.1	3.6	21.2	100	43.3	11.6	8.6	36.5	100
CT	63.2	15.9	3.4	17.5	100	38.4	12.2	8.2	41.2	100

NdfR: nitrogen derived from rhizodeposition.

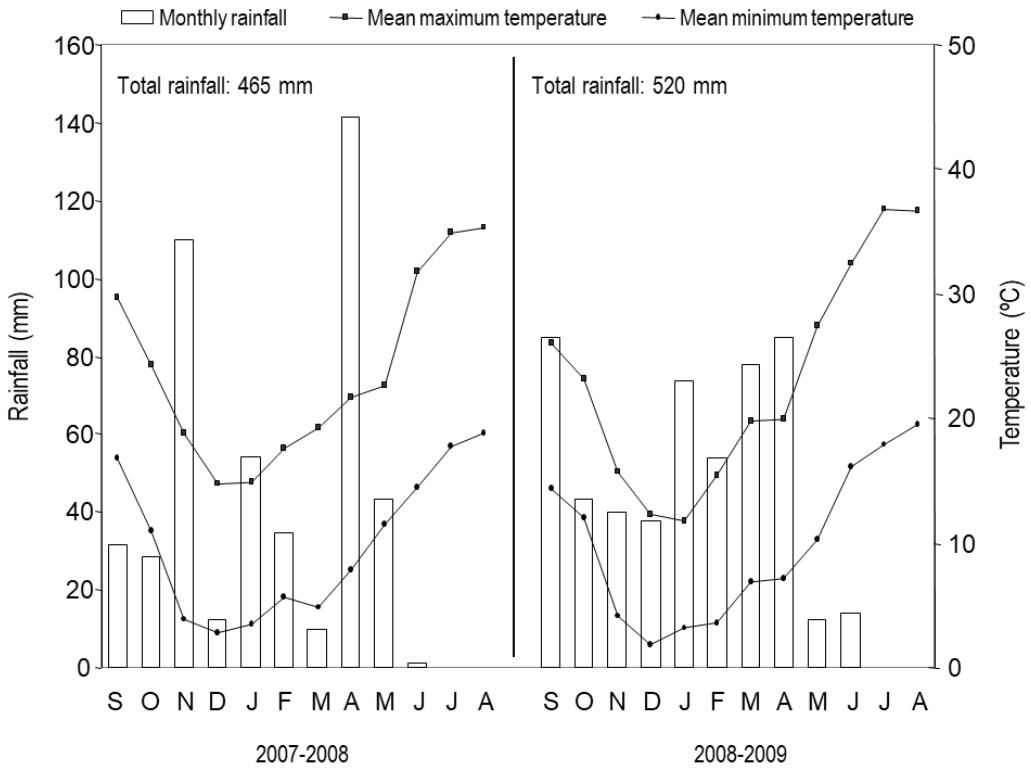

Figure 1. Monthly and annual rainfall, mean maximum and minimum temperatures over the 2-year study period at Córdoba (Spain).

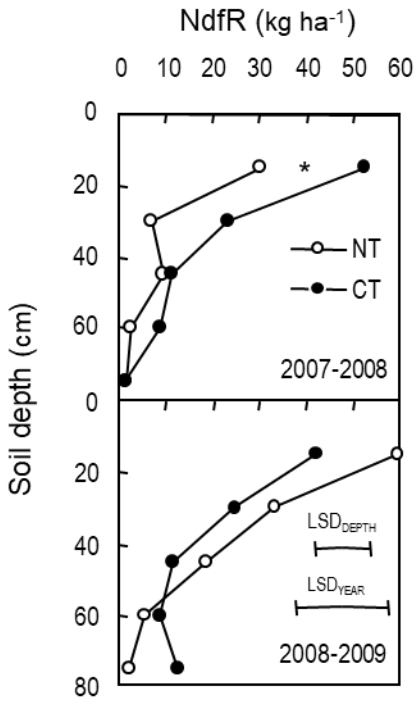

Figure 2. Nitrogen derived from rhizodeposition (NdfR) at wheat maturity as influenced by year, tillage system (NT, no tillage; CT, conventional tillage) and soil depth. NT and CT represent the total NdfR from 0-75 cm. The asterisk (*) represents a significant difference between the tillage systems. The bars represent LSD for comparison: LSD_{YEAR}, different levels of year; LSD_{DEPTH}, the same levels of year and tillage system.

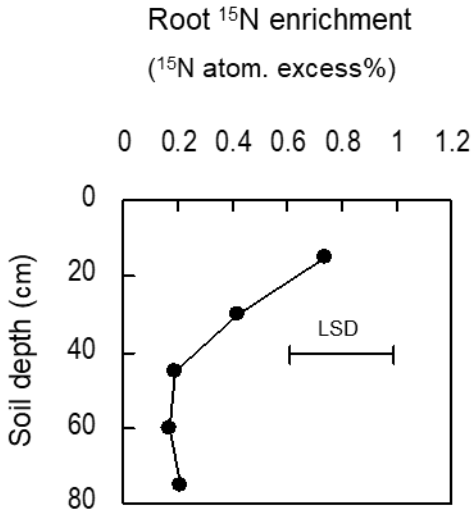

Figure 3. Root ^{15}N enrichment (average of two years) for different soil depths. Horizontal bars represent LSD ($P<0.05$) for different levels of soil depth.